

## pLV\_ Hef1a/LacO -MashI-Cerulean-Ubc-Hyg

**Backbone:** pLV-Hef1a-LacOid-NheI-Ngn3-T7-IRES2-DsRed2

**Insert:** SfiI\_ MashI-Cerulean-Ubc-Hyg\_BstEII

### Parts:

#### MashI (from pLV-TRE-MashI-IRES2-EGFP):

atggagagctctggcaagatggagagtgaggccggccagcagccgcagccccgcagcccttctgcctcccgcagc  
ctgcttctttgacaccgcccggcggcggcggcagcggcggcggcggcggcagctcagagcgcgcagcagcaacagccgc  
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cctactcctccgacgagggatcctacgaccctcttagcccagaggaacaagagctgctggactttaccaactggttc  
tga

#### Cerulean (from pPG6703 pLV UBC SfiI 6220 SfiI HEF1a Cer-2A-Puro):

atggtgagtaaaggagaagaacttttctactggagttgtcccaattcttggttgaattagatgggtgatgtaaatgggca  
caaattttctgtcagtgaggaggggtgaaggtgatgcaacatacggaaaacttacccttaaattttatgtgactactg  
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tgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttggtaataagaatcgagttaaaaggta  
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agaccattatcaacaaaatactccaattggcagatggccctgtccttttaccagacaaccattacctgtccacacaat  
ctgcccctttcgaaagatcccacgaaaagagagaccacatggtccttcttggagtttgtaacagctgctgggattaca  
ctaggcatggatgaactatacaaa

#### Ubc (from pLV-Ubc-IRES2-Hyg):

gatctggcctccgcggcgggttttggcgcctcccggggcggccccctcctcacggcgagcgcctgccacgtcagacg  
aagggcgcaggagcgtcctgatccttccgcccggacgctcaggacagcggcccgcctgctcataagactcggccttag  
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## Hyg (from pLV-Ubc-IRES2-Hyg):

atgaaaaagcctgaactcaccgcgacgtctgtcgcgagaagtttctgatcgaaaagttcgacagcgtctccgacctgat  
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atcgcccgagaagcgcggccgtctggaccgatggctgtgtagaagtaactcgccgatagtggaaccgacgccccag  
cactcgtccggatcgggaatggggggaggctaactga

## 2A:

ggatctggcgccaccaacttctctctgctgaagcaggccggcgacgtggaggagaaccaggccca

## helical linker:

ggcaacaacggcggcaacaacaacggcggctccaccggt

### - MashI forward primer (MashI\_fwd\_SfiI\_2008-06-23)

gcatgggcccattacggccgccaccatggagagctctggcaagat

### - MashI reverse primer (MashI\_rev\_helixNostop\_2008-07-17)

(fw) gctggactttaccaactggttcggcaacaacggcggcaacaacaacgg

Reverse Compliment: ccgttggttggtgcccgcgttggtgcccgaaccagttgtaaaagtcagc

### - Cerulean forward primer (Cerulean\_forward\_helix\_2008-06-27)

ggcaacaacaacggcggctccaccggtatggtgagtaaaggagaagaac

### - Cerulean reverse primer (Cerulean\_rev\_stop\_XcmI\_2008-07-08)

(fw) ctaggcatggatgaaactatacaaaataaccagggtgggattggatcgggaa

Reverse Compliment: ttcccgatccaatcccacctggttattttgtatagttcatccatgcctag

- **Ubc forward primer (Ubc\_fwd\_CerXcmIrevOverlap\_2008-07-08)**

attggatcgggaa gatctggcctccgcgcccgggt

- **Ubc reverse primer (Ubc\_rev\_kozak\_HygOverlap\_2008-07-08)**

(fw) gcttttttgttagacgaag gccgccacc atgaaaaagcctg

Reverse Compliment: caggctttttcatgggtggcggccttcgtctaacaaaaaagc

- **Hyg forward primer (Hyg\_fwd\_kozak\_UbcOverlap\_2008-07-08)**

Cgaag gccgccacc atgaaaaagcctgaactcacc

- **Hyg reverse primer (Hyg\_rev\_BstEII\_2008-07-08)**

(fw) gatgggggaggctaactgataaggtagacgtgacc

Reverse Compliment: ggtcacggtcacctcagtttagcctccccatc

**Final Construct:**

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**gaccgtgacc**